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CROP SCIENCE

Monitoring Glyphosate- and Chlorimuron-resistant *Conyza* spp. Populations in Brazil

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Abstract: *Conyza* species are important weeds in global agriculture, especially due to their capacity to evolve resistance to multiple herbicide mechanisms of action. We aimed to evaluate the frequency and distribution of resistance to glyphosate and chlorimuron-ethyl in *Conyza* spp. populations from Brazil. Seed samples were collected from grain production areas across nine Brazilian states over five consecutive years (2014 to 2018). Prior to resistance monitoring trials, dose-response assays were conducted to determine a single dose of glyphosate or chlorimuron-ethyl to discriminate resistant and susceptible populations. Resistance monitoring based on plant responses to the application of discriminatory doses of glyphosate (960 g ha^{-1}) or chlorimuron-ethyl (20 g ha^{-1}). Populations were classified as resistant, moderately resistant, or susceptible to either herbicide. While glyphosate resistance was highly frequent (71.2%) in all the five years, chlorimuron-ethyl resistant populations occurred at 39.8% of the total. The frequency of multiple resistance to both herbicides (35.3%) was proportional to the occurrence of chlorimuron-ethyl resistance (39.6%). Resistance to glyphosate and to chlorimuron-ethyl were found across all states evaluated.

Key words: multiple resistance, acetolactate synthase inhibitors, 5-enolpyruvylshikimate-3-phosphate synthase inhibitors, hairy fleabane, Sumatran fleabane.

INTRODUCTION

The *Conyza* genus belongs to the Asteraceae family and is represented by three important species for global agriculture. Horseweed (*Conyza canadensis*), hairy fleabane (*C. bonariensis*), and Sumatran fleabane (*C. sumatrensis*) are widely dispersed worldwide, causing significant yield losses in several crops (Bajwa et al. 2016). These species produce windblown seeds and are highly prolific, usually behaving as early colonizers of field margins, roadsides, and industrial areas (Dauer et al. 2007), as well as undisturbed sites such as no-till areas (Lazaroto et al. 2008). All the three species are collectively named as *Conyza* spp. or *Conyza* complex due to their morphological similarities and unclear identification.

Globally, 20, 66, and 20 unique herbicide resistance cases have been reported for *C. bonariensis*, *C. canadensis*, and *C. sumatrensis*, respectively (Heap 2021). Herbicide resistant *Conyza* spp. populations have been identified for five sites of action (SoA) in several regions across five continents. Multiple resistance evolution is the greatest concern. For instance, *C. canadensis* and *C. bonariensis* populations from the United States have evolved multiple resistance to 5-enolpyruvylshikimate-3-phosphate synthase

(EPSPS) and photosystem I (PSI) inhibitors (Moretti & Hanson 2017).

A large portion of the agricultural area in Brazil is destined for grain production. Growers usually adopt a double-cropping system in Goiás, Mato Grosso, Paraná, and Rio Grande do Sul states, in which the first crop is usually soybean cultivated in the spring/summer and the second crop is corn or cotton in the summer/fall or wheat in the winter. The *Conyza* complex commonly germinates in the fall and winter (May to October), and seed production usually occurs from December to March (Tozzi et al. 2014). The highest infestation rates are usually found in the fallow period right before soybean sowing in the spring/summer.

In 2018, more than 113 million tons of soybean were produced within 35 million ha, where more than 95% of these fields were cultivated with glyphosate-resistant (GR) varieties (Conab 2019). The repeated use of glyphosate in these areas led to the evolution of several GR weeds, including those belonging to the *Conyza* complex (Santos et al. 2014a). According to recent estimates, more than 10 million ha cultivated with soybean are currently infested with herbicide resistant *Conyza* populations in Brazil (Adegas et al. 2017). Considering that *Conyza* interference can cause up to 91% yield loss (Agostinetto et al. 2017), effective management strategies must be developed.

The first reports of GR *C. bonariensis* and *C. canadensis* populations in Brazil occurred in 2005 (Moreira et al. 2007, Lamego & Vidal 2008). Acetolactate synthase (ALS) inhibitors have been widely used to manage glyphosate resistance, which led to the evolution of populations with multiple resistance (Peterson et al. 2018). Sumatran fleabane (*C. sumatrensis*) populations in soybean and corn fields have already been reported as multiple resistant to glyphosate and chlorimuron-ethyl (Santos et al. 2014a). Multiple resistance to glyphosate, 2,4-D, saflufenacil, diuron, and paraquat has also been reported in this species (Heap 2021).

Monitoring herbicide-resistant populations provides an estimation and understanding of the problem, supporting the establishment of regional management strategies to mitigate herbicide resistance (Vargas et al. 2016). Other countries, including Canada and USA, often conduct monitoring to assess the distribution of herbicide-resistant weeds (Beckie et al. 2008, Byker et al. 2013, Matzarif et al. 2015). In Brazil, field surveys for herbicide-resistant weeds have been published for *Lolium multiflorum* (Vargas et al. 2016) and *Digitaria insularis* (Lopez Ovejero et al. 2017). However, no published studies have reported the extent and distribution of herbicide resistance in *Conyza* species. Therefore, we evaluated the distribution and frequency of glyphosate and chlorimuron-ethyl resistance in *Conyza* spp. across the major grain production areas in Brazil over five consecutive years (2014-2018).

MATERIALS AND METHODS

Nomenclature: chlorimuron-ethyl; glyphosate; *Conyza* spp.

Seed sampling and study sites

Seeds were collected from GR soybean fields where *Conyza* spp. plants survived glyphosate applications from 2014 to 2018 (January-April) in the states of Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo. Collection sites were not the same over the five years. Mature seeds were randomly collected from at least 50 plants which were then combined into a single composite sample, placed in individual paper bags and identified with their respective geographic coordinates (Burgos et al. 2013).

Dose-response

Dose-response assays were conducted to confirm that a discriminatory dose of glyphosate or chlorimuron could be used to separate resistant (R) and susceptible (S) populations. Experiments were conducted at the State University of Maringá (Maringá, Paraná, 23.40°S, 51.94°W). We selected twelve populations for dose-response studies based on a preliminary experiment (Silva et al. 2018): two from Mato Grosso and one from Goiás, Rio Grande do Sul, and São Paulo, and seven from Paraná. Experimental units consisted of pots (1 dm^3) filled with commercial potting soil (Mac Plant®, Mecpret, PR160 Road, 15, Telemaco Borba, Paraná, Brazil). Each pot received 100 seeds and plants were thinned to two per pot after emergence. Pots were irrigated with 6 mm day^{-1} and kept in the greenhouse under natural light and temperature conditions. All experiments were conducted in a completely randomized design with 4 replications. Each replication corresponded to one pot containing two plants each. In total, eight plants per population were assessed for each herbicide.

Glyphosate (Roundup Transorb R®, 480 g ae L^{-1}, Monsanto, São Paulo, Brazil) and chlorimuron-ethyl (Classic®, 250 g kg^{-1}, DuPont, São Paulo, Brazil) + mineral oil (0.5% v v^{-1}) were applied in postemergence, when plants had 5 to 6 leaves. All applications were performed from 8 to 10 am, when weather conditions were appropriate: temperature ≤ 30°C, relative humidity ≥ 60%. Herbicide treatments were sprayed with a CO_2-pressurized backpack sprayer equipped with a 2 m-long boom containing four XR 11002 nozzles (0.5 m between nozzles, TeeJet Technologies®, Cotia, Brazil). Pressure was 262 kPa, providing spray volume equivalent to 200 L ha^{-1}. Application nozzles were kept 0.5 m above the level of plant canopy.

Plants were treated with the equivalent to 0, 0.125, 0.5, 1, 2, 4 and 8-fold the recommended field dose of glyphosate (960 g acid equivalent, ae, ha^{-1}) or chlorimuron-ethyl (20 g active ingredient, ai, ha^{-1}). At 28 d after application (DAA), plant injury was evaluated using a scale from 0 to 100%, in which 0 means no injury and 100 means plant death. Plant shoot samples were also collected and dried at 60°C for 98 h before dry mass measurement.

Resistance monitoring

Greenhouse trials followed the same protocol described above and were conducted at the State University of Maringá, University of São Paulo (Piracicaba, SP, 22.70°S, 47.63°W), and University Center of Varzea Grande (Varzea Grande, MT, 15.64°S, 56.10°W). Samples were cleaned, identified, and stored as previously described. Samples were shipped to the nearest location where they were collected. Growing conditions, spraying equipment, experimental units and application stage were the same as described for the dose-response assays and across locations.

Four replications were arranged in a completely randomized design and used for each population. The experimental units were the same as for dose-response assays. Because our goal was to quantify the frequency of resistance across different fields rather than within each field, we decided to use a low number of replications to optimize the workflow with a large number of samples. While this approach allows for large scale evaluation, it can lead to uncertainties and misinterpretation of the data since a few plants are not able to represent the whole field. Another potential issue related to our data is that *Conyza* sp. identification was not performed in the field and the three species were classified as a single group. Therefore, we understand that our survey has some limitations in terms of methodology, but we still consider it as a relevant information to the discipline of weed science that will help to develop management strategies to mitigate herbicide resistance in Brazil. Pots were rearranged weekly in the greenhouse to minimize variation in light or temperature conditions. Two herbicide treatments were applied to each population: glyphosate at 960 g ae ha^{-1} or chlorimuron-ethyl at 20 g ai ha^{-1} + mineral oil (0.5% v v^{-1}). Plant injury was evaluated at 28 days after application (DAA) using the same method described above. Visual evaluations at 28 DAA were used to classify the *Conyza* populations as susceptible or resistant (Table I). This method was originally developed and proposed by Lopez Ovejero et al. (2017).

Data analysis

Dose-response data for the R and S populations were submitted to a non-linear regression model used to characterize herbicide resistance (Streibig 1988):

$$\hat{y} = \frac{a}{\left[1 + \left(\frac{x}{c}\right)^{b}\right]} \quad (1)$$

Where *y* is plant mortality or relative dry mass compared to the untreated control (dependent variable), *a* corresponds to the upper limit (asymptotic), *b* corresponds to the slope, *c* is the mean point of inflection between the upper and lower limits (50% mortality - LD_{50} or 50% relative dry mass to untreated check - GR_{50}), *d* is the lower limit and *x* is herbicide dose (independent variable). Dose-response curves

Table I. **Ranking criteria and color-coded resistance classification of *Conyza* spp. populations.**

Resistance	Mortality category	Sensitivity	Color code
Singular	All replications with control >80%	S	Green
	One or two replications with surviving plants and control <80%	r	Yellow
	Three or four replications with surviving plants and control <80%	R	Red
Multiple	All replications with control >80%	S	White
	- One or more replications with surviving plants and control <80% for chlorimuron-ethyl - All replications with surviving plants and control >80% for glyphosate	R and r-chlor	Green
	- One or more replications with surviving plants and control <80% for glyphosate - All replications with control >80% for chlorimuron-ethyl	R and r-gly	Yellow
	One or more replications with surviving plants and control <80% for both chlorimuron-ethyl and glyphosate	R and r-mult	Red

Abbreviations: S: susceptible; R: resistant; r: moderately resistant. Adapted from Lopez Ovejero et al. (2017). gly: glyphosate; chlor: chlorimuron-ethyl.

were adjusted with the SigmaPlot software (Version 12.0, Systat Software, Inc., USA) using the mean of six S and six R populations. The resistance factor (RF) was calculated using the R/S ratio for LD_{50} or GR_{50}.

In the resistance monitoring trials, populations were classified according to resistance criteria (Table I). Data were grouped by class, year, state, and relative frequencies were calculated (number of samples in each class / total number of samples) by state. Maps showing the distribution of GR *Conyza* populations were generated for each sampling year using QGis 2.14 software (QGIS, Vienna, Austria). A color-coded classification scheme was used to identify each sample, and samples were plotted on their respective geographic coordinates within the sampling site. Frequencies of susceptible, resistant and multiple-resistant populations were calculated (number of samples in each class / total number of samples).

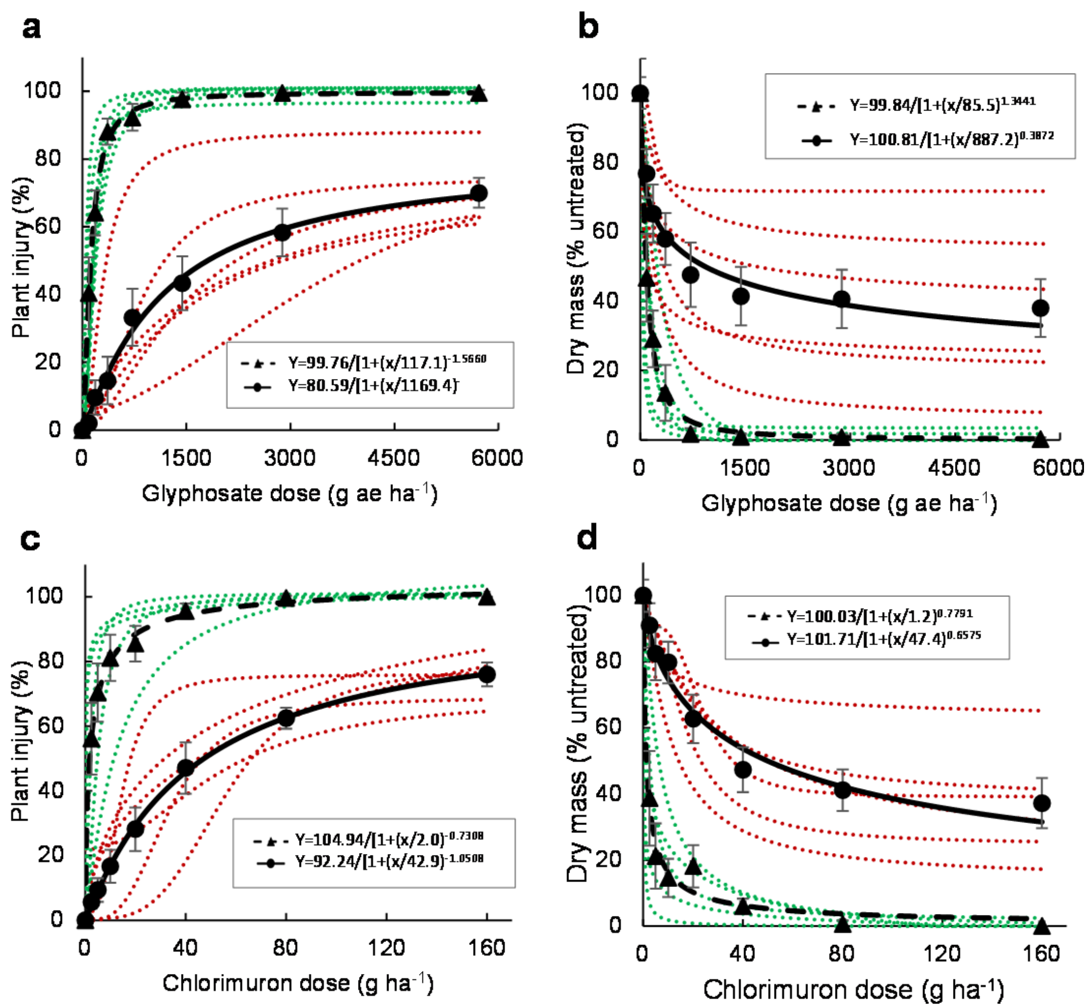

Figure 1. Glyphosate and chlorimuron-ethyl dose-response curves based on plant injury (%) and dry mass (% untreated) for 6 resistant (••••••••) and 6 susceptible (••••••••) *Conyza* populations from five Brazilian states. The non-linear regression model was adjusted ($y = a / [1+(x / c)^b]$) for resistant mean (—●—) or susceptible mean (– ▲ -). Confidence interval: 95% (n=24).

RESULTS

Dose-response assays

Replacing the independent variable by the glyphosate label dose (960 g ha^{-1}) in the adjusted equations, the R populations showed mean mortality levels of 35.8% and mean mass reduction of 46.6%. The S populations presented mean mortality of 96.2% and mass reduction of 96.3%. The label dose for chlorimuron-ethyl (20 g ha^{-1}) provided 29.4% mortality for R populations, compared to 88.5% for S populations. Similarly, 35.1% of dry mass reduction would be expected for R populations and 89.7% for S populations. These values demonstrate that the recommended field doses for each herbicide allow for the discrimination of S and R populations using 80% of control or 80% of dry mass reduction as discriminant values.

The non-linear regression model for dose-response based on plant mortality (Figure 1) indicated RF = 10-fold for glyphosate and 21.5-fold for chlorimuron-ethyl. Based on relative dry mass the R population was 10.4-fold and 38.3-fold more resistant than the S population to glyphosate and chlorimuron-ethyl, respectively. Those numbers confirm the high level of multiple resistance to glyphosate and chlorimuron-ethyl in *Conyza* populations.

Resistance monitoring

In total, 1184 samples were collected and screened over the five years (265 in 2014, 219 in 2015, 206 in 2016, 282 in 2017, and 213 in 2018). Paraná State had the highest sampling number, in which 43.3, 71.6, 53.4, 52, and 52.8% of the total populations were sampled in 2014, 2015, 2016, 2017, and 2018, respectively.

Most areas sampled in Brazil demonstrated high resistance frequencies in the first year. S populations did not exceed 39% of total samples, whereas R populations and moderately resistant (r) accounted for at least 61.5% in each year (Table II). There was a slight increase in the S to R ratio over the years, especially in 2017 and 2018, probably due to an increased number of samples that were collected from states where the frequency of S populations was relatively high, such as Goiás, Santa Catarina and Mato Grosso (Figure 2). Populations classified as R or r to glyphosate were found in all sampled states in at least one out of the five years (Figure 1 and Table III).

There was a high frequency (71.2%) of GR (R or r) when the combined data from all years (from 2014 to 2018) were examined (Table IV). The states with the lowest frequencies of GR (R or r) populations were Santa Catarina and Rio Grande do Sul, whereas the highest frequencies were found in Paraná, Mato Grosso do Sul, Minas Gerais, and Bahia (Table IV). Nonetheless, Rio Grande do Sul is known to have an increasing number of sites with low performance of glyphosate in *Conyza* populations, especially in the Western and Northern regions of the state (Vargas et al. 2010).

As a result of low seed germination in some samples, a total of 1,119 samples were tested for chlorimuron-ethyl resistance, 65 less than those evaluated for glyphosate. The states of Paraná and Minas Gerais showed the highest number of samples classified as R to this herbicide. Resistance to chlorimuron-ethyl was relatively low (≤19.3%), except in 2015, when 38.1% of the samples were ranked as R (Table II). S populations were found in high frequencies in 2016 and 2017, while fewer populations were ranked as R and r in those years (Table II and Figure 3). Most resistance cases were found in Paraná, but resistant populations had already been found in Bahia (2014, 2015 and 2016), Mato Grosso (all years), and Rio Grande do Sul (all years), which implies a wide geographic distribution of resistance to chlorimuron-ethyl in Brazil. Under the current

Table II. Frequency (%) of *Conyza* spp. populations classified as susceptible (S), moderately resistant (r), and resistant (R) to glyphosate and chlorimuron-ethyl in Brazil.

Herbicide	Class	2014	2015	2016	2017	2018
Glyphosate	S	7.2	10.5	16.5	35.9	38.5
	r	17.0	10.0	10.2	13.9	13.1
	R	75.8	79.5	73.3	50.2	48.4
	Total	100	100	100	100	100
Chlorimuron-ethyl	S	47.1	35.9	70.9	80.4	55.2
	r	41.1	26.0	15.9	9.8	25.5
	R	11.8	38.1	13.2	9.8	19.3
	Total	100	100	100	100	100

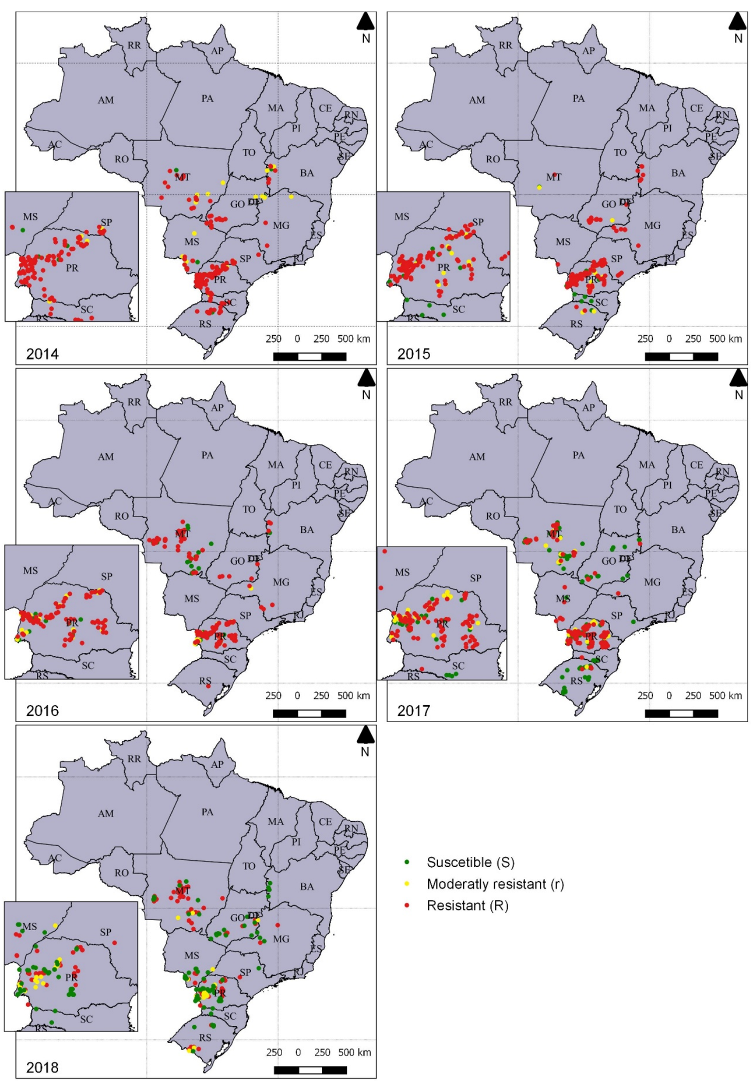

Figure 2. Distribution of *Conyza* spp. populations resistant to glyphosate in Brazil between 2014 and 2018.

Table III. Frequency (%) of *Conyza* spp. populations with multiple resistance to glyphosate and chlorimuron-ethyl in Brazil.

Class of resistance to			2014	2015	2016	2017	2018
Glyphosate	Chlorimuron-ethyl	Glyphosate and chlorimuron-ethyl					
S	S	S gly and chlor	4.9	9.9	13.8	32.5	31.9
S	r	r-chlor	1.9	1.1	0.0	1.8	5.6
S	R	R-chlor	0.4	1.1	0.0	1.8	0.9
r	S	r-gly	8.7	5.5	9.5	9.1	7.0
r	r	r-mult	7.2	2.2	1.1	3.3	4.2
r	R	R-mult	0.8	2.2	0.5	1.5	1.9
R	S	R-gly	33.5	20.4	47.6	39.1	16.0
R	r	R-mult	31.9	22.7	14.8	4.7	15.5
R	R	R-mult	10.6	34.8	12.7	6.2	16.4
Total			100	100	100	100	100

Abbreviations: S: susceptible; r: moderately resistant; R: resistant.

Table IV. Number (N°) and frequency (%) of *Conyza* spp. populations resistant (R) or moderately resistant (r) to glyphosate and chlorimuron-ethyl by state sampled in 2014, 2015, 2016, 2017, and 2018.

State	Glyphosate			Chlorimuron-ethyl			Multiple		
	N°	Σ (R+r)	% (R+r)	N°	Σ (R+r)	% (R+r)	N°	Σ (R+r)†	% (R+r)
BA	34	26	76.5	25	10	40.0	25	8	32.0
GO	68	48	70.6	62	31	50.0	62	28	45.2
MT	265	188	70.9	241	75	31.1	241	75	31.1
MS	47	34	72.3	47	26	55.3	47	21	44.7
MG	25	19	76.0	20	8	40.0	20	7	35.0
PR	625	534	85.4	616	260	42.2	616	249	40.4
SP	38	32	84.2	27	8	29.6	27	8	29.6
SC	20	10	50.0	19	4	21.1	19	4	21.1
RS	62	34	54.8	62	29	46.8	62	24	38.7
Total	1184	925	71.2	1119	451	39.6	1119	424	35.3

†(R + r to glyphosate) + (R + r to chlorimuron-ethyl). Σ: sum.
Abbreviations: BA: Bahia, GO: Goiás, MT: Mato Grosso, MS: Mato Grosso do Sul, MG: Minas Gerais, PR: Paraná, SP: São Paulo, SC: Santa Catarina, RS: Rio Grande do Sul.

relatively low frequency of R populations, those ranked as R to chlorimuron-ethyl were concentrated in Western Paraná (Figure 3), where resistance was reported for the first time (Santos et al. 2014a). The highest number of samples ranked as R or r to chlorimuron-ethyl were found in Mato Grosso do Sul, Goiás, Rio Grande do Sul, Paraná, Bahia and Minas Gerais. In the remaining states, the frequency of R or r samples was lower than 40% (Table IV).

Multiple resistance to glyphosate and chlorimuron-ethyl formed a peak of frequency in 2017 for S populations (32.5%). However, the frequency of multiple resistance in populations ranked as S or r populations was relatively low (<10%) in all years, since most samples were considered R to chlorimuron-ethyl. The frequency of multiple resistance (R to glyphosate and R to chlorimuron-ethyl) samples was low in all years, except in 2015, when a high frequency of chlorimuron-ethyl resistance increased the number of populations with multiple resistance (Table IV and Figure 4).

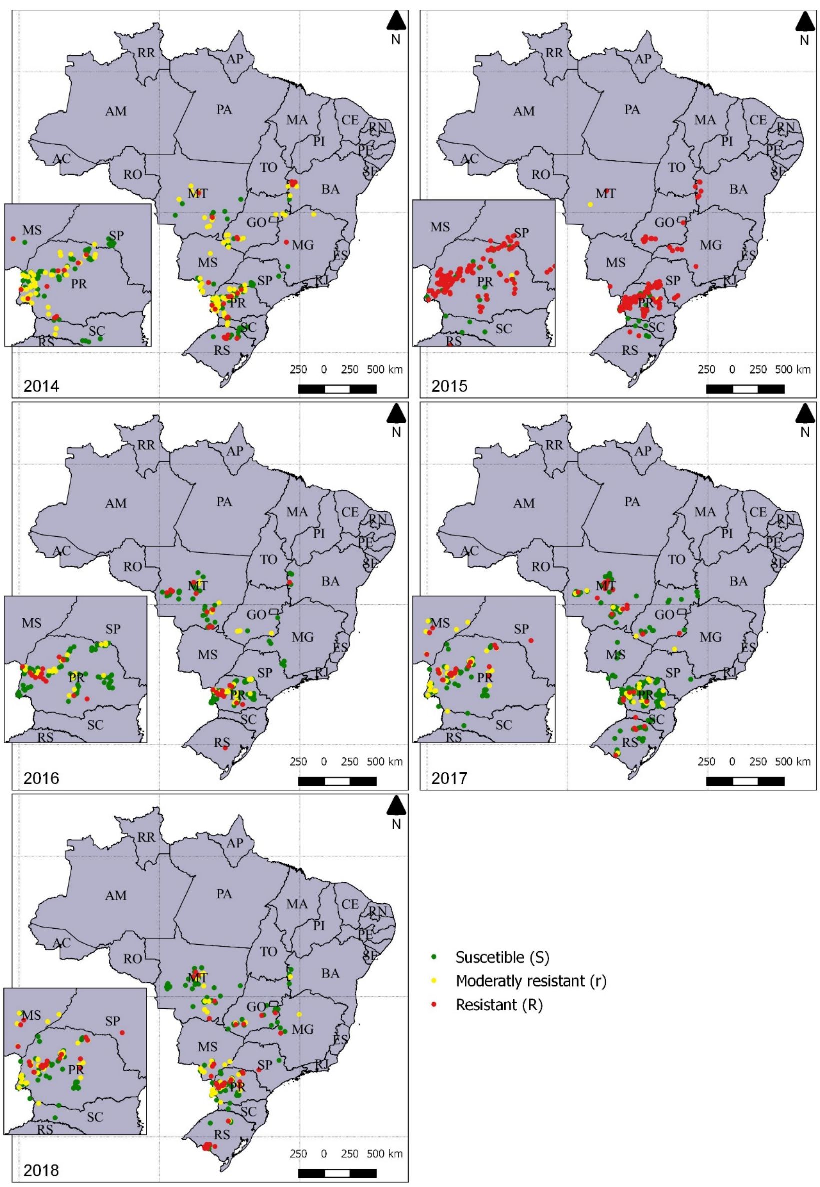

Figure 3. Distribution of *Conyza* spp. populations resistant to chlorimuron-ethyl in Brazil between 2014 and 2018.

DISCUSSION

Herbicide resistance surveys conducted in Australia for *Raphanus raphanistrum* and *Lolium rigidum* evaluated 80 and 40 plants per population, respectively (Walsh et al. 2007, Owen et al. 2014). We conducted a large-scale survey of multiple herbicide resistance in *Conyza* species across nine different states. In our study, we decided to use eight plants per population to optimize sample testing even though this is less than what other researchers have used. While large-scale studies provide useful information for weed management, they require high efficiency for sample testing. Although our data demonstrate the spread of resistant populations in multiple regions of the country, eight plants can be insufficient to estimate the frequency of resistant individuals within a population.

The doses used in the monitoring trials for glyphosate and chlorimuron-ethyl resistance were enough to control S populations, and provide less than 80% control on R plants. These doses were used to discriminate S and R *Conyza*

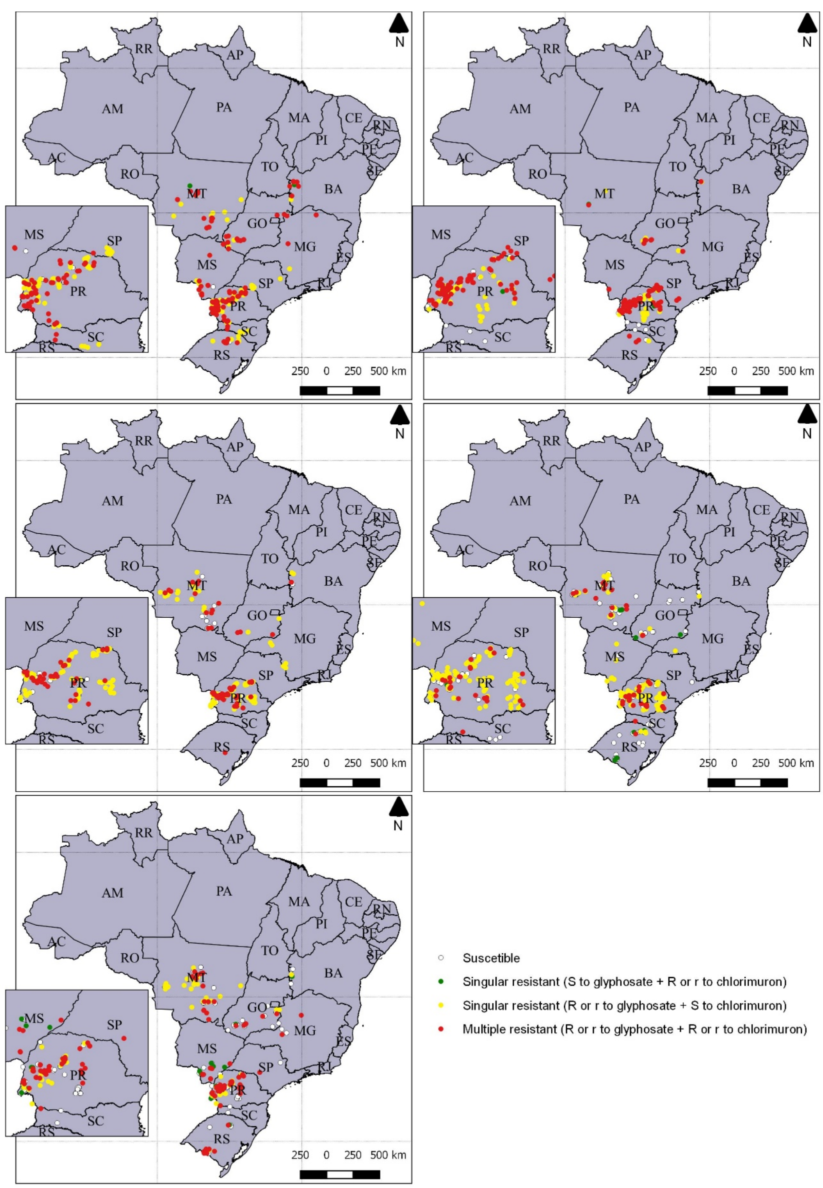

Figure 4. Distribution of *Conyza* spp. populations with multiple resistance to glyphosate and chlorimuron-ethyl in Brazil between 2014 and 2018. R: resistant; r: moderately resistant; S: susceptible.

spp. populations in several other publications elsewhere (Lamego & Vidal 2008, Santos et al. 2014a, b). Herbicide sensibility can vary among different species from the same genus. The doses used in our research (960 g ha^{-1} for glyphosate and 20 g ha^{-1} for chlorimuron) have shown to be able to discriminate S and R populations in the three *Conyza* spp. species (Kruger et al. 2009, Santos et al. 2014a, Puricelli et al. 2015).

In a field survey from different regions of Indiana (USA), GR *C. canadensis* populations were found in all sampled areas (Davis et al. 2008). In the same state, 63% of the total assessed populations were resistant to glyphosate and 20% to chlorimuron-ethyl, but only 2% of the populations were resistant to both herbicides simultaneously (Kruger et al. 2009). In the present study, the combined results from all years indicate that 35.3% of the samples were multiple resistant, while 39.6% were resistant to chlorimuron-ethyl only (Table IV). Therefore, the frequency of multiple resistance is proportional to the frequency chlorimuron-ethyl resistance since most populations are already resistant to glyphosate. Even though chlorimuron-ethyl is an effective alternative option for managing glyphosate resistant weeds, these *Conyza* populations have not been under intense selection pressure as high as glyphosate. This might help to explain why a high number of chlorimuron-ethyl susceptible populations were still observed.

Species from the *Conyza* genus have high levels of genetic variability and molecular evidence shows that *C. canadensis*, *C. bonariensis*, and *C. sumatrensis* share a common ancestral genome (Soares et al. 2015, Marochio et al. 2017). Thus far, only *C. sumatrensis* populations have been identified as resistant to chlorimuron-ethyl, but populations of *C. canadensis* and *C. bonariensis* may also be evolving chlorimuron-ethyl resistance, since the species were not identified in this research. *Conyza* species have up to 4% of cross pollination, and 2.5% of pollen deposition was found as far as 480 m downwind from the source edge (Huang et al. 2015). The sum of these biological characteristics can contribute to the evolution of herbicide resistance.

The first two cases of GR *Conyza* in Brazil were almost simultaneously found in orange orchards from São Paulo (Moreira et al. 2007) and in soybean fields from Rio Grande do Sul (Lamego & Vidal 2008). Apparently, from 2007 to 2014, either enough time elapsed for the spread of resistant populations (gene flow and seed dispersion) or independent selection occurred in different places due to the repeated use of glyphosate. Wind is the main mechanism for *Conyza* dispersion (Dauer et al. 2007, Ye et al. 2016), whereby seeds can travel distances as far as 500 km (Shields et al. 2006). Less than one year after the first case of glyphosate resistance in *C. canadensis* was reported in Canada, resistant populations were found 400 km away from the original site (Byker et al. 2013). Wind seed dispersal indicates that resistance is not only related to selection pressure by repeated herbicide use but also to intense seed production and availability of large amounts of seeds for wind dispersal across different fields. Seed movement through combines and planting machines may also contribute to gene flow and hence to resistance spread. For sourgrass (*Digitaria insularis*), another GR species in Brazil, two main aspects are responsible for the fast spread of resistant populations: the migration of rented combines from Southern Brazil towards the Midwest and Northeast regions, and the local selection in response to the intense use of glyphosate (Takano et al. 2018).

Following the confirmation of glyphosate resistance in different *Conyza* species in Brazil, ALS-inhibiting herbicides such as chlorimuron-ethyl became important tools to manage weeds

(Oliveira Neto et al. 2013, Peterson et al. 2018). The first chlorimuron-ethyl-resistant populations were found in Western Paraná in 2012 (Santos et al. 2014a). Recently, populations of *C. sumatrensis* with multiple resistance to three different mechanisms of action (glyphosate, EPSPS inhibitor; chlorimuron-ethyl, an ALS inhibitor; and paraquat, a PSI inhibitor) were also reported in Paraná, and another population was identified as resistant to saflufenacil (PPO inhibitor) (Heap 2021). These findings indicate that lack of control with glyphosate and chlorimuron-ethyl are continuously increasing, leading to the use of other herbicides and, consequently, resistance evolution to other mechanisms of action, including PSI and PPO inhibitors.

The rapid widespread distribution of herbicide resistant *Conyza* throughout different regions of Brazil is probably associated with seed movement by equipment or wind, and/or with independent selection for resistance. Alternative approaches aiming to prove local solutions and prevent the selection and widespread of herbicide resistance in *Conyza* spp. should be developed and implemented. This initiative should not be restricted to glyphosate and chlorimuron-ethyl resistance, but also to prevent multiple herbicide resistance evolution. More importantly, nonchemical weed management tools such as crop rotation can also support growers to control the *Conyza* complex in their fields. For instance, recent research indicated that the use of ruzigrass (*Urochloa ruziziensis*) as a cover crop during fallow periods can suppress up to 80% of emergence of *C. sumatrensis* (Marochi et al. 2018).

While this survey was able to map where resistant populations are present across different agricultural regions, we emphasize that the assessment of low number of replications could lead to uncertainties and misinterpretation of the data. In addition, considering the three different species as the same Conyza complex could increase variability in the response to each herbicide. Therefore, we understand our survey may have limitations in terms of methodology, but we still believe that our data can contribute to the discipline of weed science and must help to mitigate herbicide resistance in Brazil.

CONCLUSIONS

This work provides evidence that glyphosate resistance in the *Conyza* complex is frequent and widespread across all grain-producing areas of Brazil. Most samples in this work were ranked as chlorimuron-ethyl susceptible, but resistant populations were identified in particular sites dispersed throughout all sampled states. The frequency of populations with multiple resistance to both herbicides was proportional to the occurrence of chlorimuron-ethyl resistance.

Acknowledgments

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